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Evaluation of sealed vacuum extraction method (Seditainer) for measurement of erythrocyte sedimentation rate

W N PATTON, P J MEYER, J STUART* **Department of Haematology, Medical School, University of Birmingham, and the Department of Haematology, Queen Elizabeth Hospital, Birmingham*

SUMMARY A sealed vacuum extraction method (Seditainer) for determining the erythrocyte sedimentation rate (ESR) was compared with the standard Westergren ESR technique. The Seditainer method was particularly easy to use, showed acceptable precision, reduced the biohazard risk to laboratory staff and, on storage of sealed blood specimens for 24 hours at 4°C, gave ESR values that had decreased by a mean of only 1·9% (95% CI +0·2 to −4·0%). Seditainer tubes are shorter (100 mm) than Westergren tubes (200 mm) and this reduced test sensitivity at ESR values above 55 mm/first hour. After application of the manufacturer's correction formula to adjust for tube length there was improved correlation ($n = 150$, $r = 0{\cdot}936$, $p < 0{\cdot}001$), but still considerable scatter, between the Seditainer and Westergren results. Seditainer ESR values above 55 mm/first hour should therefore be regarded as semiquantitative.

This vacuum extraction method offers a simple and safe technique for measuring the ESR and specimens can be stored overnight at 4°C.

Introduced in 1921, the erythrocyte sedimentation rate (ESR) remains the most widely used laboratory method for monitoring the acute phase response in inflammatory disorders.[1,2] The method is particularly suited to monitoring relatively long term (> 24 hours) changes in the plasma concentration of large molecular weight acute phase proteins such as fibrinogen and some immunoglobulins.[3] The selected ESR method of the International Committee for Standardization in Haematology (ICSH)[4] is based on that of Westergren.[2]

Many variations of the ICSH selected method are used but comparability and quality control of the procedure remain poor. Several ESR methods also pose a biohazard risk. A new sealed ESR system (Seditainer) relies on direct vacuum extraction of the patient's blood into a sterilised tube of siliconised glass containing anticoagulant which itself forms the ESR tube and is disposable.[5] As the specimen tube is not opened before disposal the biohazard risk to the phlebotomist and laboratory staff is reduced. Other potential advantages include enhanced precision resulting from controlled dilution of blood with anticoagulant-diluent solution and the potential to store blood specimens before testing. We therefore evaluated the Seditainer ESR system and compared it with the standard Westergren method.

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Material and methods

Venous blood was taken from hospital patients, using a butterfly needle (21 gauge; Abbott Laboratories, Sligo, Eire). Blood (5 ml) was aspirated manually into 1·5 mg/ml of K_2EDTA for analysis by Westergren ESR. A Vacutainer Multiple Sample Adapter was then fitted to the butterfly tubing and 5·2 ml of blood was aspirated by vacuum into 1·3 ml trisodium citrate (105 mmol) in a Seditainer tube (Becton Dickinson Vacutainer Systems, Oxford).

The Westergren ESR was performed according to the ICSH recommendations[4] but using a plastic disposable 200 mm ESR tube (Sterilin Ltd, Feltham, Middlesex) to reduce the biohazard risk. This method correlated closely ($r = 0{\cdot}997$, $p < 0{\cdot}001$) with the ICSH method using glass tubes (BS 2554; J Bibby Science Products, Stone, Staffordshire) for 40 hospital patients with ESR values in the range 1–154 mm/first hour. Dilution of blood, anticoagulated with K_2EDTA, in trisodium citrate was performed carefully using a micropipette. The Westergren tube was mounted vertically within two hours of venepuncture unless otherwise stated. Simultaneously, the vacuum aspiration tubes were placed vertically in the manufacturer's stand and the ESR values read on a linear scale at 60 minutes. Where stated, Seditainer ESR values were converted to Westergren reference

Table 1 *Reproducibility of volume of blood aspirated into anticoagulant-diluent in 25 Seditainer tubes as determined by the increase in weight of each tube and final PCV and red cell count**

		Seditainer blood samples
Increase in weight (g)	Mean	5·197
	CI†	5·123–5·261
	CV%	3·0
PCV*	Mean	0·353
	CI	0·348–0·358
	CV%	3·4
Red cell count* ($\times 10^{12}$/l)	Mean	4·16
	CI	4·10–4·22
	CV%	3·7

*The instrument CV for 25 measurements on a single normal blood was 0·5% for PCV and 0·6% for red cell count.
†95%.

values (corrected Seditainer ESR) using a conversion table provided by the manufacturer.

Results

REPRODUCIBILITY OF BLOOD SAMPLING AND DILUTION

To determine the precision of vacuum aspiration of 5·2 ml blood 25 Seditainer tubes were weighed before and after sequential collection of blood at one venepuncture and the packed cell volume (PCV) and red cell count determined by Coulter S880 counter (Coulter Electronics Ltd, Luton, Bedfordshire). The results indicate good accuracy and precision in vacuum aspiration and pre-dispensed volume of citrate and thus in dilution of blood (table 1).

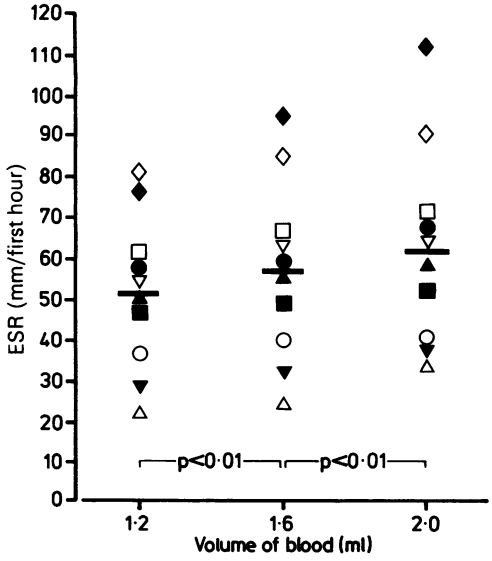

Fig 1 *Effect on Westergren ESR of 10 patients, of varying volume of anticoagulated blood added to 0·4 ml citrate diluent. Significance determined by Wilcoxon signed rank test for paired data.*

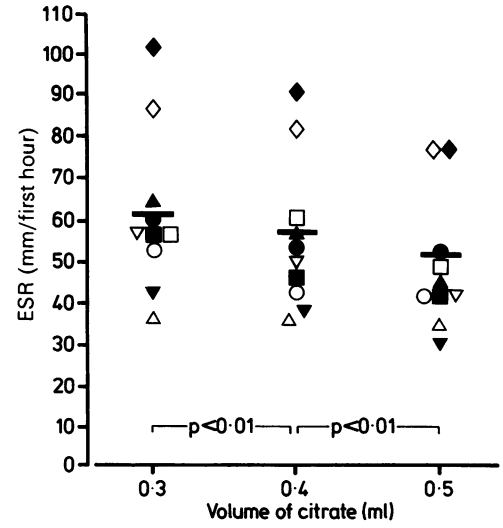

Fig 2 *Effect on Westergren ESR of 10 patients, of varying volume of citrate diluent to which was added 1·6 ml anticoagulated blood. Significance determined by Wilcoxon signed rank test for paired data.*

Blood samples for estimation of Westergren ESR require to be diluted accurately (within 5%) by adding four parts of blood to one part of citrate diluent.[4] When performed in the laboratory this dilution can be made with precision. Some recently introduced ESR systems reduce the biohazard risk to laboratory staff by providing an ESR sample tube containing pre-dispensed anticoagulant diluent, the tube being filled with blood to a mark on the label by the phlebotomist. To show the potential inaccuracy of this approach, the optimal volume of 1·6 ml blood was deliberately

Table 2 *Effect of variation in internal diameter of disposable Westergren ESR tubes from each of four manufacturers on ESR of 40 patients*

	Manufacturer			
	A	*B*	*C*	*D*
Internal diameter (mm) (n = 20)				
Mean	2·40	2·50	2·59	2·68
CI†	2·37–2·42	2·48–2·51	2·57–2·61	2·66–2·71
CV%	1·7	1·1	1·6	2·0
ESR (mm/first hour) (n = 40)				
Mean	66·7	62·3	59·4	57·0
SEM	6·4	6·0	6·0	6·0

†95%.

Table 3 *Within batch precision of uncorrected and corrected Seditainer and Westergren ESR methods for 10 replicate measurements on normal blood with added gelatin and on blood from two patients*

	Erythrocyte sedimentation rate (mm/first hour)			
	Mean	*95% CI*	*Range*	*CV (%)*
Added gelatin:				
1 mg/ml:				
Seditainer uncorrected	12·3	12·0–12·6	12–13	3·9
Seditainer corrected	14·3	14·0–14·6	14–15	3·4
Westergren	12·0	11·4–12·6	11–14	6·8
2 mg/ml:				
Seditainer uncorrected	41·8	41·5–42·1	41–42	1·0
Seditainer corrected	58·8	58·5–59·1	58–59	0·7
Westergren	49·8	48·8–50·8	48–52	3·0
Patients' blood:				
Case 1:				
Seditainer uncorrected	13·0	12·7–13·3	12–14	3·6
Seditainer corrected	15·1	14·6–15·6	14–17	4·9
Westergren	15·4	14·9–15·9	14–16	4·5
Case 2:				
Seditainer uncorrected	52·6	51·8–53·4	51–54	2·0
Seditainer corrected	80·6	78·7–82·5	77–84	3·3
Westergren	65·8	63·0–68·6	60–72	5·9

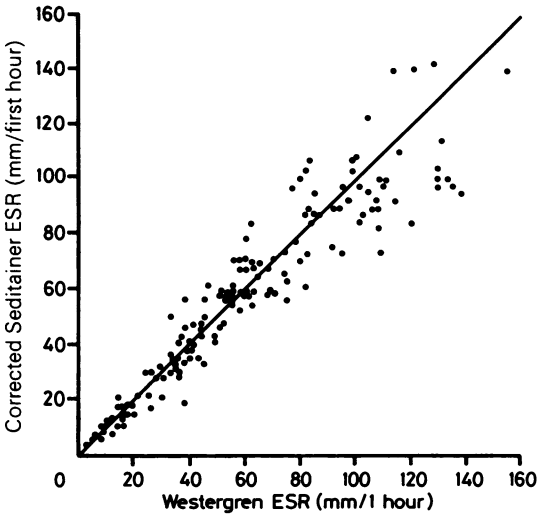

Fig 4 *Scattergram and 45° line showing the same data as in fig 3 but with Seditainer ESR adjusted for tube length, using manufacturer's conversion table, to give corrected Seditainer ESR values ($r = 0{\cdot}936, p < 0{\cdot}001$).*

varied when added to edetic acid tubes containing 0·4 ml of 109 mmol/l trisodium citrate. The results indicate a 10% increase in mean Westergren ESR when there was 25% overfilling (2 ml rather than 1·6 ml) of the specimen tube (fig 1). When a standard volume (1·6 ml) of edetic acid-anticoagulated blood from 10 patients was added to a volume of citrate that varied around the optimal volume of 0·4 ml there was a 7% increase in ESR when there was a 25% decrease (from 0·4 to 0·3 ml) in volume of diluent (fig 2).

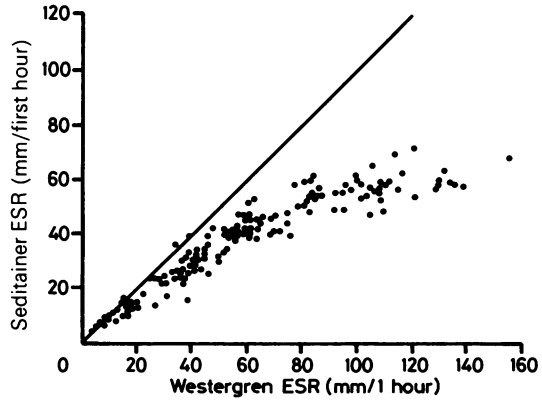

Fig 3 *Scattergram and 45° line showing correlation between uncorrected Seditainer and Westergren ESR values for 150 specimens ($r = 0{\cdot}927, p < 0{\cdot}001$).*

INTERNAL DIAMETER OF WESTERGREN TUBES

The mean internal diameter of 20 disposable Westergren ESR tubes from each of four manufacturers was calculated from the volume of water aspirated to the 200 mm mark of each tube, the individual tubes being weighed before and after filling. The coefficient of variation for the calculated internal diameter of 20 tubes ranged from 1·1–2·0% for the four manufacturers (table 2). When the ESR of 40 patients (range 4–154 mm/first hour) was measured using 40 tubes from each manufacturer, the mean ESRs were found to vary inversely with tube diameter (table 2). Two way analysis of variance showed a highly significant ($p < 0{\cdot}001$) variation in ESR between the four manufacturers' tubes, almost all of this variation (98%) being accounted for by linear regression on the tube diameter. After allowing for this regression variation in ESR between tubes was no longer significant.

PRECISION

The precision of corrected Seditainer and Westergren methods was determined using normal blood with the addition of 1 mg/ml or 2 mg/ml 5% w/v gelatin (Sigma Chemical Co, St Louis, Missouri, USA) and a final haematocrit of 0·35, to give raised ESR values (table 3). A 100 ml venous blood sample from each of two patients with a raised ESR was also studied. Ten different Seditainer tubes were filled and 10 Westergren ESR dilutions made from each of the above four specimens and the ESRs then measured. The co-

Table 4 *ESR measurements before and after storage of 100 specimens for 24 hours at room temperature and 4°C*

	Storage temperature	Mean (SEM) ESR (mm/first hour)		Mean (95% CI) change in ESR (mm/first hour)		(%)	
		0 hours	24 hours	Mean	CI	Mean	CI
Uncorrected Seditainer ESR	RT*	41·3 (1·6)	32·8 (1·7)	− 8·5	− 6·4 to −10·6	−20·6	−15·5 to −25·7
(range 4–72 mm/first hour)	4°C	—	40·5 (1·6)	− 0·8	+ 0·1 to − 1·7	− 1·9	+ 0·2 to − 4·0
Corrected Seditainer ESR	RT*	62·4 (3·0)	47·6 (3·0)	−14·8	−11·1 to −18·5	−23·7	−17·8 to −29·7
(range 4–161 mm/first hour)	4°C	—	60·8 (3·0)	− 1·5	+ 0·2 to − 3·2	− 2·4	+ 0·2 to − 5·1
Westergren ESR	RT*	63·5 (3·1)	44·6 (3·1)	−18·9	−14·6 to −23·2	−29·8	−23·1 to −36·5
(range 3–131 mm/first hour)	4°C	—	59·5 (3·2)	− 4·0	− 2·0 to − 6·0	− 6·3	− 3·1 to − 9·5

*RT, room temperature.

efficient of variation (CV) for 10 replicate measurements ranged from 1·0–3·9% for the uncorrected Seditainer ESR, 0·7–4·9% for the corrected Seditainer ESR, and 3·0–6·8% for the Westergren ESR (table 3).

CORRELATION BETWEEN SEDITAINER AND WESTERGREN ESRS

One blood sample from each of 150 patients was studied using both ESR methods. There was a linear correlation ($n = 74$, $r = 0{\cdot}937$, $p < 0{\cdot}001$) between uncorrected Seditainer and Westergren methods for ESR values up to 55 mm/first hour (fig 3). Above this level uncorrected Seditainer ESR values showed a limited further rise (up to 70 mm/first hour) as the Westergren ESR increased to 155 mm/first hour. The correlation between methods in the range 56–155 mm/first hour was ($n = 76$, $r = 0{\cdot}778$, $p < 0{\cdot}001$).

Seditainer ESR values were then adjusted for the short length of tube (100 mm effective length compared with 200 mm for Westergren tubes) using the manufacturer's conversion table. Although there was an improved correlation and linear relation between the corrected Seditainer and Westergren ESR values, there was considerable scatter (fig 4).

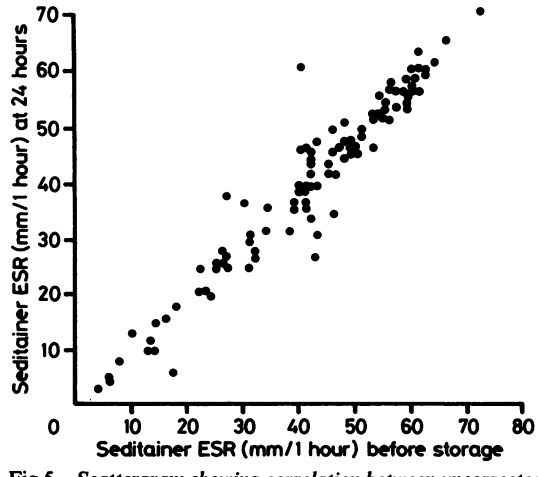

Fig 5 *Scattergram showing correlation between uncorrected Seditainer ESR values before and after storage of 100 blood specimens at 4°C for 24 hours ($r = 0{\cdot}963$, $p < 0{\cdot}001$).*

STABILITY OF BLOOD SAMPLE

The stability of Seditainer and Westergren ESR blood specimens from 100 patients was determined before and after 24 hours' storage at room temperature or 4°C; blood for the Westergren ESR was stored in its original tube containing edetic acid without added citrate. Specimens stored at 4°C were allowed to equilibrate to room temperature over 45 minutes before performing the ESR. All specimens were mixed for 10 minutes on a roller mixer before setting up the ESR.

Storage at room temperature was unsatisfactory for both methods (table 4) but specimens stored at 4°C showed a mean fall of only 1·9% for uncorrected, and 2·4% for corrected, Seditainer ESR values compared with 6·3% for Westergren ESR samples. The highly significant correlation ($r = 0{\cdot}963$, $p < 0{\cdot}001$) between uncorrected Seditainer ESR values before and after storage for 24 hours at 4°C is shown in fig 5).

Discussion

The closed vacuum extraction technique of the Seditainer reduces the biohazard risk to the phlebotomist and to laboratory staff. As the unopened blood specimen tube also serves as the ESR tube measurement of the ESR is particularly easy.

Westergren ESRs require dilution of four parts blood to one part of citrate diluent and this dilution step is a potential cause of poor quality control. This is particularly so when the phlebotomist expresses blood directly from a syringe to a marked level in a specimen tube containing pre-dispensed citrate diluent. Such tubes are often overfilled, causing a falsely high ESR. Similarly, if there is loss by leakage or evaporation of the pre-dispensed citrate diluent in a manufacturer's tube a falsely high ESR will result. As the ratio of blood to citrate increases, the diluting effect of the

anticoagulant on the plasma concentration of rouleaux-inducing proteins is decreased so that more rouleaux form and the ESR increases. Our data confirm the potential error of manual dilution of blood into pre-dispensed citrate at the bedside. In the Seditainer system the 4:1 dilution is performed automatically by vacuum extraction and this proved to be reproducible and should contribute to better precision.

It is known that the Westergren ESR is affected by the diameter of the tube and ICSH specifies an internal diameter of 2·4–2·7 mm.[4] We have now shown that, even within this range, the ESR depends on diameter so that the ICSH range would seem to be too wide. Seditainer tubes are of much wider bore (9 mm) which reduces dependency on diameter and should improve precision between laboratories at the cost of a larger sample volume (5·2 ml) of blood.

The shorter (100 mm) length of the Seditainer tube is a considerable disadvantage as false low ESR values are obtained above 55 mm/first hour. Although the conversion table provided by the manufacturer allowed partial correction for this, there was still considerable scatter between Seditainer and Westergren results. Thus Seditainer values should be considered as semiquantitative at ESR values above 55 mm/first hour. Our Seditainer ESRs were read using a stand with a linear scale. An alternative stand with a non-linear scale, which avoids the need for the conversion table, is available from the manufacturer but it is intrinsically less accurate to read high ESRs from such a non-linear scale.

A major limitation of Westergren ESR methods is the need to set up the ESR tube within two hours of venepuncture when blood is stored at room temperature, or within six hours when stored at 4°C.[4] A sealed vacuum extraction technique, using tubes and citrate diluent that are bacteriologically sterile, has potential for longer storage, and a mean fall in the ESR of only 1·9% was found after 24 hours' storage at 4°C. This permits a useful extension of the six hours' storage at 4°C approved by ICSH.[4]

A further development of sealed vacuum extraction ESR systems is indicated in view of the benefits of simplicity, safety, and potential for overnight storage.

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Requests for reprints to: Professor J Stuart, Department of Haematology, The Medical School, University of Birmingham, Birmingham B15 2TJ, England.